**Cooperation Centre for Scientific Research Relative to Tobacco**

**Smoke Analysis Sub-Group**

# CORESTA Recommended Method No. 106

# DETERMINATION OF HYDROGEN CYANIDE IN MAINSTREAM CIGARETTE SMOKE BY CONTINUOUS FLOW ANALYZER

February 2024

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**Status:** Valid

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# CORESTA RECOMMENDED METHOD N° 106

## DETERMINATION OF HYDROGEN CYANIDE IN MAINSTREAM CIGARETTE SMOKE BY CONTINUOUS FLOW ANALYZER

(February 2024)

## 0. INTRODUCTION

At the outset of this work, discussions in the CORESTA Smoke Analysis Sub-Group determined that most laboratories would use a method involving a Continuous Flow Analyzer for the determination of hydrogen cyanide (HCN) in mainstream cigarette smoke because they considered it most suitable and this was therefore chosen as the basis of the Recommended Method. The method involved smoke collection using a combination of either a) one glass fiber filter pad followed by one impinger trap containing dilute sodium hydroxide solution or b) one ethanol/water sodium hydroxide-treated glass fiber filter pad followed by one blank pad. HCN was determined using a Continuous Flow Analyzer based on the detection of the coloring system formed by the reaction of cyanides with some chromogenic reagents, such as isonicotinic acid/1,3-dimethyl barbituric acid and pyridine/pyrazolone.

Initial joint experiments and on-going discussions addressed some methodological aspects that needed to be considered before moving to a Recommended Method. The Recommended Method was produced through a final collaborative study involving 18 laboratories from five countries. Measurement data using both ISO 3308 and ISO 20778 are also provided in the 2022 collaborative study report. This method includes notes to inform other laboratories that might wish to adopt it about some of the main features that need to be well controlled to provide data as robust and consistent as the repeatability and reproducibility data provided. Statistical evaluations were made according to ISO recommendations and are included.

## 1. SCOPE

This Recommended Method is applicable to the determination of hydrogen cyanide in mainstream cigarette smoke, using a Continuous Flow Analyzer.

## 2. NORMATIVE REFERENCES

**2.1** ISO 3308, *Routine analytical cigarette-smoking machine – Definitions and standard conditions*

**2.2** ISO 20778, *Cigarettes – Routine analytical cigarette smoking machine – Definitions and standard conditions with an intense smoking regime*

**2.3** ISO 3402, *Tobacco and tobacco products – Atmosphere for conditioning and testing*

**2.4** ISO 4387, *Cigarettes – Determination of total and nicotine-free dry particulate matter using a routine analytical smoking machine*

**2.5** ISO 8243, *Cigarettes – Sampling*

## 3. TERMS AND DEFINITIONS

No specific terms and definitions are listed in this document**.**

## 4. PRINCIPLE

Cigarettes are smoked on a standard smoking machine that has been fitted with a glass fibre filter (Cambridge Filter Pad, CFP) followed by an impinger (trapping system 1) or by a combination of CFP treated by ethanol – water sodium of hydroxide solution and a blank glass fibre filter (trapping system 2). HCN collected on pads is then extracted with sodium hydroxide solution. HCN in the CFP extract and impinger are determined using a Continuous Flow Analyzer based on the detection of the coloring system formed by the reaction of cyanides with chromogenic reagents, such as isonicotinic acid/1,3-dimethyl, barbituric acid and pyridine/pyrazolone. The absorbance is detected at 600 nm.

## 5. APPARATUS AND EQUIPMENT

### 5.1 Normal laboratory apparatus and equipment is needed, in particular, the following items

- Equipment needed to perform conditioning of tobacco products
- Equipment needed to perform marking for butt length
- Equipment needed to perform smoking of tobacco products complying with ISO 3308 or ISO 20778
- Tubing (e.g. Nalgene) 1/4” ID X 3/8” OD
- Soap bubble flow meter to measure puff volume
- Leak tester to ensure no air leaking of smoking machine

### 5.2 The necessary general laboratory equipment for the preparation of samples, standards, and reagents

- Analytical balance, capable of measuring four decimal places
- Dispenser capable of delivering volume of 50 mL
- Triangular flask (e.g., 150 mL, 500 mL)
- Impinger for trapping mainstream smoke (e.g., 70 mL, 250 nominal volume)
- Volumetric flask with ground glass joint (e.g., 50 mL, 100 mL)
- Transfer pipettes (100 µL, 1000 µL, 5 mL, 10 mL)
- Magnetic stirrer and stir bars
- Sample cups for autoanalyzer
- Wrist-action shaker
- 5 $cm^3$ disposable syringe
- Syringe filter (0,45 µm, PES or equivalent)
- Glass fibre filter pad

**5.3 Computer-controlled Continuous Flow Analyzer (or equivalent) consisting of:**

- Technicon I Autosampler.
- Technicon II Peristaltic Pump.
- Technicon III Chemistry Manifold.
- Technicon IV Colorimeter (with 600 nm filter).

## 6. REAGENTS AND SUPPLIES

All reagents shall be, at the least, recognized as analytical reagent grade. Specific reagents may vary based on Continuous Flow Analyzer equipment used.

**6.1** Chloramine T (CAS 127-65-1) ≥ 98,0 % purity

**6.2** Potassium biphthalate (CAS 877-24-7) ≥ 99,5 % purity

**6.3** 1,3-Dimethylbarbituric acid (CAS 769-42-6) ≥ 98,0 % purity

**6.4** Isonicotinic acid (CAS 55-22-1) ≥ 99,0 % purity

**6.5** Polyoxyethylene lauryl ether (Brij-35) (CAS 9002-92-0) ≥ 90,0 % purity

**6.6** Anhydrous ethanol (CAS ) ≥ 99,0 % purity

**6.7** Sodium hydroxide (CAS 1310-73-2) ≥ 95 % purity

**6.8** Certified stock sodium cyanide (e.g., 50,0 mg/L)

**NOTE:** The use of this method can involve hazardous materials, operations and equipment. This method does not purport to address all safety problems associated with its use. It is the responsibility of the user of this method to establish appropriate safety and health practices and determine the applicability of regulatory limitations prior to use.

## 7. PREPARATION OF SOLUTIONS (Recommended)

### 7.1 Brij 35 solution (25 %)

Add 1 L deionized water to 250 g Brij 35, warm and stir until dissolved.

### 7.2 Chloramine–T solution

Dissolve 2.0 g chloramine-T, weighed to 0,01 g, in 500 mL of deionized water. Mix well. This solution shall be stored at 4 °C and prepared monthly, as required.

### 7.3 Buffer solution

Dissolve 2,3 g sodium hydroxide and 20,5 g potassium biphthalate, weighed to 0,01 g, in 1 L of deionized water. Add 0,5 mL of Brij-35 solution and mix well. This solution shall be stored at 4 °C and prepared monthly, as required.

### 7.4 Chromogenic reagent solution

Dissolve 7,0 g sodium hydroxide, 16,8 g 1,3-dimethylbarbituric acid and 13,6 g isonicotinic acid, weighed to 0,01 g, in 1 L of deionized water. Add 0,5 mL of Brij-35 solution and mix well. This solution shall be stored at 4 °C and prepared monthly, as required.

**NOTE:** The pH of buffer solution and chromogenic reagent solution should be adjusted to 5.3 by sodium hydroxide and hydrochloric acid solution.

### 7.5 Ethanol - water solution of sodium hydroxide (1 M)

Dissolve 4.0 g sodium hydroxide pellets in 50 mL of deionized water and then add the same volume of ethanol (50 mL) into the water solution. Mix well. Store at room temperature and prepare monthly, as required.

### 7.6 Sodium hydroxide solution (0.1 M)

Dissolve 8 g sodium hydroxide pellets in 2 L of deionized water. Stir until completely dissolved. Store at room temperature.

## 8. PREPARATION OF STANDARDS

### 8.1 Primary (1°) stock standard

Certified stock sodium cyanide, of an appropriate concentration, shall be obtained from a commercial supplier.

### 8.2 Working standards

Take appropriate volumes (0,10 mL to 6,0 mL, for example) of the 1° stock standard and dilute to the prescribed volumes with 0,1 M sodium hydroxide solution to prepare at least five working standards. For example, 0,100; 0,500; 1,000; 3,000; 4,000; 6,000 mg/L of working standards were prepared by diluting 0,1; 0,5; 1,0; 3,0; 4,0; 6,0 mL aliquots of 1° stock standard (50 mg/L). Store at 4 °C and prepare weekly, as required.

**NOTE:** The calibration should cover the concentration range of interest.

## 9. SAMPLING

As applicable, perform sampling in accordance with ISO 8243.

## 10. TOBACCO PRODUCT PREPARATION

Condition the cigarettes in accordance with ISO 3402.

## 11. SAMPLE GENERATION

### 11.1 Smoking machine setup

The smoking parameters for which the method has been studied are set out in ISO 3308 and in ISO 20778 as shown in Table 1.

**Table 1 - Cigarette Smoking Parameters Employed**

| Smoking regime | Puff volume (mL) | Puff frequency (seconds) | Puff duration (seconds) | Ventilation Blocking (%) |
|---|---|---|---|---|
| ISO 3308 | 35 | 60 | 2 | 0 |
| ISO 20778 | 55 | 30 | 2 | 100 |

## 11.2 Trapping Systems

Two different trapping systems can be either used. Trapping system 1 consists of a CFP combined with an impinger. Trapping system 2 consists of a treated and a blank CFP.

### 11.2.1 Trapping System 1 – CFP and Impinger

Assemble the HCN mainstream apparatus on the smoking machine by connecting the cigarette holder with one CFP and a 70 mL fritted stem impinger containing 30 mL 0,1 M sodium hydroxide solution for ISO 3308 and a 250 mL fritted stem impinger containing 90 mL 0,1 M sodium hydroxide solution for ISO 20778 (Figure 1).

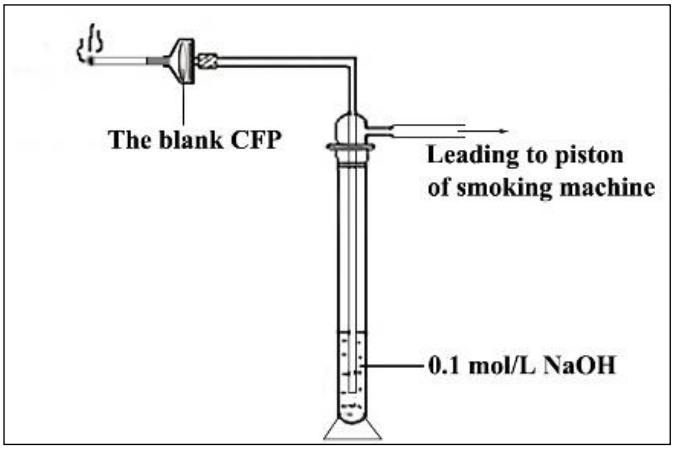



**Figure 1 - Example of a suitable Trapping System 1**

**NOTE:** It is recommended that the trapping efficiency be checked when validating this method under both the ISO 3308 and ISO 20778 smoking regimes. To check the trapping efficiency of the method, add an additional impinger and follow the method accordingly. Analyse each impinger individually for the compounds of interest. If no compounds are detected in the additional impinger, only the prescribed number of impingers is then required to trap the vapour phase HCN effectively, otherwise an additional impinger is required.

### 11.2.2 Trapping System 2 – Treated CFP and blank CFP

Impregnate a CFP with 1,0 M ethanol-water solution of sodium hydroxide and place together with a blank CFP in a filter holder connected to the smoking machine. The steps to prepare the CFP impregnation and assembling of the trapping system are described below:

#### 11.2.2.1 Pre-treatment of CFP

- Linear smoking machine: place a 44 mm conditioned CFP into a culture disk (diameter: 60 mm) or a beaker containing 2,0 mL ethanol - water solution of sodium hydroxide. After being fully soaked, it is put into a fume hood for about ten minutes to evaporate ethanol and then transferred into the laboratory conditioner and conditioned for 1 h to 3 h at 22 °C ± 1 °C and 60 % ± 2 % of relative humidity.
- Rotary smoking machine: place a 92 mm conditioned CFP into a culture disk (diameter: 100 mm) or a beaker containing 8,0 mL ethanol - water solution of sodium hydroxide. After being fully soaked, it is put into a fume hood for about ten minutes to evaporate ethanol and then transferred into the laboratory conditioner and conditioned for 1~3 h at 22 °C ± 1 °C and 60 % ± 2 % of relative humidity.

#### 11.2.2.2 Assembly of Trapping System 2

- Place the treated CFP and a blank CFP into the cigarette holder.
- The treated CFP should be in the front facing the incoming smoke and the blank CFP on the back.
- All the rough sides of both CFPs should face the incoming smoke.
- Assemble the cigarette holder in the smoking machine and connect the rear section of cigarette holder to the tubing leading to the piston of the smoking machine.
- Examine the system with a leak tester to ensure no air leaking is present.

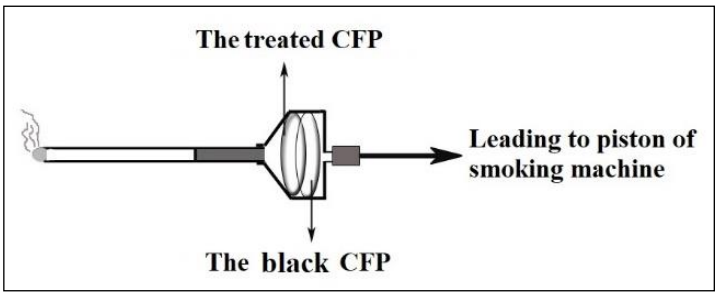



**Figure 2 - Example of Trapping System 2**

### 11.3 Cigarette smoking

The cigarettes are smoked according to ISO 4387 with the following modifications:

- Check the puff volume of each port and adjust accordingly.
- Typically 4 cigarettes are smoked per replicate for ISO 3308 and 2 cigarettes per replicate for ISO 20778 smoking regime using a linear smoking machine. If using a rotary smoking machine, the number of cigarettes smoked per replicate should be increased.

## 12. SAMPLE ANALYSIS

### 12.1 Extraction from Trapping System 1

- After smoking, remove the pad, fold into quarters (with the condensate inside) and wipe the inside of the holder with the pad.
- For the 44 mm CFP, place the pad into a 100 mL triangular flask containing 50 mL of 0,1 M sodium hydroxide solution. For a 92 mm CFP, place the pads into a 500 mL triangular flask containing 100 mL of 0,1 M sodium hydroxide solution.
- Shake the triangular flasks at 200 rpm for 30 min on the wrist action shaker. The extract was filtered into a sample cup by 0,45 μm membrane filter.
- Transfer the impinger solution into a volumetric flask (e.g. 100 mL, 200 mL). Rinse the impinger with 0,1 M sodium hydroxide solution with appropriate volumes (for example: 10 mL for 70 mL impinger, 30 mL for 250 mL impinger) three times and add to the volumetric flask to the nominal volume. Transfer the solution to the sample cup.

## 12.2 Extraction from Trapping System 2

- After smoking, remove the two pads together from their holder, fold into quarters (with the condensate inside) and wipe the inside of the holder with the pad.
- Place the pads into a 150 mL triangular flask containing 100 mL 0,1 M sodium hydroxide solution. For the 92 mm CFP, place the pads into a 500 mL triangular flask containing 250 mL 0,1 M sodium hydroxide solution.
- Shake the triangular flasks at 200 rpm for 30 min on the wrist action shaker. The extract was filtered into the sample cup by 0,45 μm membrane filter.

## 12.3 Continuous flow analysis (Example)

Examples of detection conditions for analysis of samples obtained from both trapping systems are shown in the paragraphs below. The examples shown are applicable for "Seal AA3" equipment [1].

### 12.3.1 Conditions for continuous flow analysis (Example)

- Sample time: 60 s.
- Wash time: 60 s.
- Aspirate the system with deionized water (with 1 mL of Brij 35 added into 1 L deionised water) for approximately 30 minutes.
- Transfer the reagent pickup tubes to their appropriate reagents (APPENDIX A) and aspirate until a steady baseline and uniform bubble patterns are observed.
- The bubbles should flow smoothly through the Continuous Flow Analyzer and be uniform in shape and spacing with rounded ends.
- The auto-sampler is operated at a sampling rate of 30 per hour with a 1:1 sample to wash ratio. Sufficient time should be required for the system to become stable with the reagents being pumped.

### 12.3.2 Calibrations

- This calculation is performed automatically by the analytical instrument. Plot a graph of corrected absorbance units for each standard against HCN content (APPENDIX B). The calibration graph should have an $R^2$ value of >0,999. The response obtained for test samples should fall in the range of the calibration curve.

### 12.3.3 Determination of Response Factor

- A calibration curve for HCN is prepared by plotting the concentration of the standards versus the peak height to determine appropriate response factors.
- The concentration of HCN in mainstream smoke is quantified by the external standard method.
- Sample quantification

[1] This example is given for the convenience of the users of this Recommended Method and does not constitute an endorsement of this instrument.

- The amount of HCN in mainstream smoke per cigarette trapped by CFP is calculated as follows:

$$M_{CFP} = \frac{1{,}038 \times C \times V}{N}$$

Where

$M_{CFP}$ is the amount of HCN in mainstream smoke trapped by CFP per cigarette, expressed in μg/cigarette;

*1,038* is the conversion coefficient of cyanide ion to hydrogen cyanide;

*C* is the response factor of HCN in CFP derived from the calibration curve, expressed in μg/mL;

*V* is the volume of CFP extract, expressed in mL;

*N* is the number of cigarettes smoked per CFP.

- The amount of HCN in mainstream smoke trapped by impinger is calculated as follows:

$$M_{impinger} = \frac{1{,}038 \times C \times V}{N}$$

Where

$M_{impinger}$ is the amount of HCN in mainstream smoke per cigarette trapped by impinger, expressed in μg/cigarette;

*1,038* is the conversion coefficient of cyanide ion to hydrogen cyanide;

C is the response factor of cyanide ion in impinger derived from the calibration, expressed in μg/mL;

V is the final volume of impinger solution, expressed in mL;

N is the number of cigarettes smoked per impinger.

**NOTE:** For the trapping system 1, the total HCN in mainstream smoke per cigarette shall be the combination of HCN trapped by CFP and impinger. For the trapping system 2, the total HCN in mainstream smoke per cigarette shall be the HCN trapped by CFP.

## 13. REPEATABILITY AND REPRODUCIBILITY

The full collaborative study was conducted in 2022[1], involving 18 laboratories and five replicate analyses of 7 cigarette samples including the University of Kentucky reference cigarettes 1R6F and 2R5F and the CORESTA Monitor Test CM9 and 4 commercial cigarettes as shown in Table 2. Repeatability and reproducibility were reported in absolute values and as a percentage of the mean. Statistical evaluation for the 2022 collaborative study carried out followed the methods provided by ISO 5725-2[2] to generate repeatability (r) and reproducibility (R) data on a combined data set. Repeatability ranged between 7,34-19,98 % of the mean and reproducibility between 13,56-32,30 % of the mean. The r & R results for each product under the two smoking regimes are presented in Table 3.

**Table 2 - Cigarette Samples Included in the Joint Experiment**

| Sample | Description | ISO 3308 'tar' level (mg/cig) |
|---|---|---|
| KR 2R5F | American Blend | 1 |
| Sample 1 | American blend – charcoal filter | 3 |
| Sample 2 | American blend – charcoal filter | 5 |
| KR 1R6F | American Blend | 8 |
| Sample 3 | Virginia Blend | 8 |
| Sample 4 | Virginia Blend | 11 |
| CM9 | Virginia Blend | 16 |

**Table 3 - Repeatability and Reproducibility**

| Smoking Regime | Sample | N of data | Mean | r (µg/cig) | r (%) | R (µg/cig) | R (%) |
|---|---|---|---|---|---|---|---|
| ISO 3308 | CM 9 | 21 | 139,34 | 12,33 | 8,85 | 26,69 | 19,15 |
| | KR 1R6F | 19 | 95,67 | 11,47 | 11,99 | 16,92 | 17,69 |
| | KR 2R5F | 19 | 12,82 | 2,56 | 19,98 | 4,14 | 32,30 |
| | Sample 1 | 20 | 26,96 | 4,29 | 15,93 | 7,99 | 29,63 |
| | Sample 2 | 19 | 53,54 | 7,46 | 13,94 | 11,54 | 21,56 |
| | Sample 3 | 19 | 83,5 | 8,77 | 10,5 | 19,86 | 23,79 |
| | Sample 4 | 19 | 103,54 | 11,01 | 10,63 | 17,18 | 16,59 |
| ISO 20778 | CM 9 | 20 | 302,7 | 27,53 | 9,09 | 51,53 | 17,02 |
| | KR 1R6F | 20 | 394,9 | 44,49 | 11,27 | 101,16 | 25,62 |
| | KR 2R5F | 16 | 377,04 | 27,66 | 7,34 | 55,69 | 14,77 |
| | Sample 1 | 19 | 324,66 | 28,1 | 8,66 | 81,54 | 25,11 |
| | Sample 2 | 20 | 337,91 | 35,9 | 10,63 | 85,39 | 25,27 |
| | Sample 3 | 19 | 265,71 | 23,68 | 8,91 | 65 | 24,46 |
| | Sample 4 | 17 | 248,68 | 21,97 | 8,84 | 33,71 | 13,56 |

Where r is repeatability, R is reproducibility

## 14. REPORT

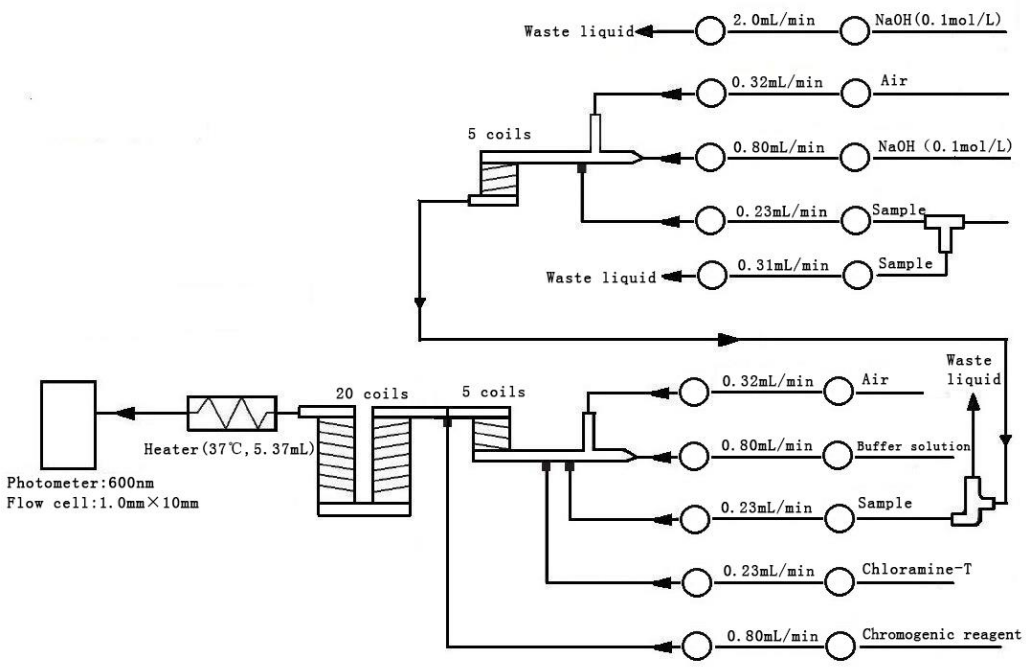
The report shall state the amount of HCN in micrograms per cigarette. HCN yields in the mainstream smoke of cigarette in µg/cigarette were rounded to the nearest 0,01 µg. The report shall include all conditions not specified in this Recommended Method or regarded as optional that may have influenced the results. The report shall also give all details necessary for the identification of each sample.

## 15. BIBLIOGRAPHY

[1] CORESTA Technical Report, 2022 Collaborative Study for HCN in Mainstream Cigarette Smoke – February 2024 [SA-296-2-CTR]

[2] ISO 5725-2:1994: Accuracy (trueness and precision) of measurement methods and results – Part 2: Basic method for the determination of repeatability and reproducibility of a standard measurement method.

## APPENDIX A – THE MANIFOLD OF CONTINUOUS FLOW ANALYZER FOR HYDROGEN CYANIDE DETECTION




## APPENDIX B – TYPICAL GRAPH OF HYDROGEN CYANIDE DETERMINATION BY CONTINUOUS FLOW ANALYZER